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AN UNUSUAL PATTERN OF GENE FLOW BETWEEN THE TWO SOCIAL FORMS OF THE FIRE ANT *SOLENOPSIS INVICTA*

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Abstract.—Uncertainty over the role of shifts in social behavior in the process of speciation in social insects has stimulated interest in determining the extent of gene flow between conspecific populations differing in colony social organization. Allele and genotype frequencies at 12 neutral polymorphic protein markers, as well as the numbers of alleles at the sex-determining locus (loci), are shown here to be consistent with significant ongoing gene flow between two geographically adjacent populations of *Solenopsis invicta* that differ in colony queen number. Data from a thirteenth protein marker that is under strong differential selection in the two social forms confirm that such gene flow occurs. Data from this selected locus, combined with knowledge of the reproductive biology of the two social forms, further suggest that interform gene flow is largely unidirectional and mediated through males only. This unusual pattern of gene flow results from the influence of the unique social enviroments of the two forms on the behavior of workers and on the reproductive physiology of sexuals.

Key words.—Fire ants, gene flow, monogyny, polygyny, social forms, *Solenopsis invicta*.

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Considerable controversy exists over the role that changes in social organization play in initiating reproductive isolation and ultimately in driving speciation in social insects. Significant modifications to colony social organization, such as a shift from monogyny (single reproductive queen per colony) to polygyny (multiple reproductive queens per colony), are thought in some instances to promote modification of mating systems such that reproductive isolation may develop between the parental and derived social forms (Crozier 1977; Elmes 1978; Brian 1983; West-Eberhard 1986). Evidence for this scenario in ants comes from the widespread occurrence of sets of closely related species or conspecific "forms" that differ in colony queen number (Brian and Brian 1955; Wilson 1971; Ross et al. 1987; Ward 1989; Hölldobler and Wilson 1990; Frumhoff and Ward 1992). These related social variants often differ in important properties of their breeding biology that may be expected to restrict gene flow between them, such as the site of mating or mode of colony founding (Keller and Passera 1989; Buschinger 1990; Bourke and Franks 1991; Elmes 1991; Keller and Ross 1993a).

Two general approaches can be taken to assess the importance of shifts in colony social organization in promoting cladogenesis in social insects. One is to track the phylogenetic distribution of social organization. Discovery of a recurring pattern in which sister species exhibit divergent social organizations presumably would support a major role for social evolution as a driving force in speciation (see Ward 1989). Another approach is to conduct detailed genetic studies of paired conspecific populations possessing alternative forms of social organization. If shifts in social organization frequently initiate the development of reproductive isolation, then some such pairs are expected to occupy an intermediate stage of evolutionary divergence that is marked by restricted gene flow combined with a lack of morphological distinctiveness (Ross 1988).

The fire ant *Solenopsis invicta* is a social insect with two distinct population types that differ in colony social organization but not in taxonomically informative morphological features. Thus, it is a candidate for the latter approach to studying the relationship between social evolution and speciation. Both monogyne and polygyne social forms occur in the native range of this species in South America, as well as in its introduced range in the southeastern United States (Glancey et al. 1973; Fletcher et al. 1980; Ross and Fletcher 1985a; Ross et al. 1993). Polygyne colonies in the United States occur usually in discrete, persistent populations that are embedded in large populations of the more common monogyne form (Ross et al. 1987; Mackay et al. 1991; Porter 1992; but see also Porter et al. 1991). The social forms of *S. invicta* differ not only in queen number, but in other important reproductive traits

such as mode of colony founding and pace of queen reproductive development (Vargo and Porter 1989; Glancey and Lofgren 1988; Porter 1991; Keller and Ross 1993a). Monogyne queens participate in mating flights and found their new colonies independently (without the assistance of workers). Polygyne queens take part in mating flights or may mate in the nest (Ross and Fletcher 1985b; Porter 1991), but these queens always initiate reproduction in established nests (Glancey and Lofgren 1988; Porter 1991). Realization that the two social forms have diverged in some important elements of their breeding biology has led to the suggestion that they are reproductively isolated and in the process of speciating (Ross and Fletcher 1985a; Ross et al. 1987). Thus, it is important to determine whether the two social forms of *S. invicta* are linked by ongoing gene flow and if so to establish the magnitude of this gene flow and the manner in which it is effected.

The ability to survey many polymorphic genetic markers in *S. invicta* (Ross et al. 1993; Shoemaker et al. 1992) allows us to examine these issues in detail for a single pair of geographically adjacent monogyne and polygyne populations from northern Georgia, United States. The data presented here are consistent with the occurrence of extensive gene flow between these populations, and furthermore they suggest that such gene flow is largely unidirectional and mediated through males only.

Materials and Methods

Sample Collections

Twenty-nine nests of polygyne *Solenopsis invicta* were collected from Walton County, Georgia, United States and returned to the laboratory for extraction from the soil and collection of samples for electrophoretic analysis. Collected nests were separated by a minimum distance of 10 m to reduce the chance that any pair belonged to the same polydomous colony (Vargo and Porter 1989). Polygyny has been documented previously at the Walton County site (Fletcher 1983; Ross and Fletcher 1985a) and was confirmed in each of these 29 sampled nests by (1) collecting multiple reproductive queens in each nest and dissecting them to confirm that they were mated, and (2) inspecting genotypes of nestmates at 11 polymorphic protein loci to establish that these individuals represented multiple matrilines.

Live polygyne queens ($n = 172$) were collected from 53 nests in this same population and were separated individually into small rearing units with workers and brood to track their brood production patterns in the laboratory. Queens that produce numerous sons among their offspring under such conditions are known to be diploid-male-producing queens (Ross and Fletcher 1985b; Ross et al. 1993). The proportion of such queens among all mated polygyne queens was determined by inspecting brood patterns 6 wk after queens were isolated to obtain a measure of allelic diversity at the sex-determining locus (loci). Source nests were confirmed to be polygyne by virtue of multiple fertile queens having been collected from each.

Fifty-two nests of monogyne *S. invicta* were collected from several counties adjacent to the polygyne study population (Putnam, Jasper, and Morgan counties, Georgia) to provide material for electrophoretic analysis. All of these colonies were located within 100 km of the polygyne study population, and most were considerably closer. Monogyny, which was inferred initially on the basis of worker size and mound distribution (Greenberg et al. 1985), was confirmed in all of these colonies by inspecting nestmate genotypes for at least one (and usually several) of the polymorphic protein markers. Predictable patterns in the identities and observed segregation ratios of offspring genotypes occur in monogyne fire ant colonies because each foundress queen mates only once (e.g., Ross and Fletcher 1985a; Shoemaker et al. 1992).

Live, newly mated monogyne queens ($n = 648$) were collected immediately after their mating flights from the same area in which the monogyne nests were collected. The queens were held individually in small rearing cups in the laboratory and their brood production was tracked to estimate the proportion of diploid-male-producing queens (production of sexual larvae and male pupae in the first brood is diagnostic of such queens in this form, see Ross and Fletcher 1985b, 1986). All of these newly mated queens were individually weighed to confirm that they originated from monogyne nests; sexually mature nonreproductive queens of the monogyne form consistently are heavier than their polygyne counterparts (Ross and Fletcher 1986; Porter et al. 1988; Keller and Ross 1993a).

Estimation of the Number of Sex Alleles

Queens of *S. invicta* that produce diploid males do so because they have had a "matched mating." That is, they have mated with one or more

(haploid) males that have an allele in common with them at the sex-determining locus (loci) (Adams et al. 1977). Because queens from the two study populations effectively mate only once (e.g., Ross and Fletcher 1985a), the proportion of diploid-male-producing queens is equivalent to the proportion of matched matings (Θ) in these populations. The effective number of alleles (K) segregating at a single sex-determining locus under strong frequency-dependent selection is a simple function of the proportion of matched matings:

$$K = 2/\Theta$$

(Adams et al. 1977). Extension to a two-locus system, in which the loci are assumed to be in linkage equilibrium and to each have the same number of alleles present at equal frequencies, leads to

$$K = [(4 \cdot K + 4)/\Theta]^{1/3},$$

where K is now the effective number of alleles segregating at either locus (Ross et al. 1993). The 95% confidence intervals about the estimates of Θ and K were obtained by drawing 200 bootstrap samples from the original data sets, estimating Θ for each sample, and eliminating the 5 extreme high and 5 extreme low bootstrap estimates (e.g., Weir 1990).

Electrophoresis

Individual genotypes were scored at 13 polymorphic protein-encoding loci following electrophoresis in horizontal starch gels and specific staining (see Ross 1992; Shoemaker et al. 1992). Source material for electrophoresis varied according to the particular marker (see table 1). Although many of the markers can be scored from more than one life stage or caste, all comparisons in this study except those for *Pgm-3* were confined to data derived from a single type of source material for each marker.

Genotypic data for all markers except *Pgm-4* were obtained from all of the polygyne nests; this marker was not scored in three of the nests. From 7 to 37 individual genotypes were scored per polygyne nest for any given locus. In most instances, only subsets of the 52 monogyne colonies were used to generate genotypic data; colonies composing these subsets were chosen largely because they contained an abundance of a particular life stage or caste. From 6 to 55 individual genotypes were scored per monogyne nest for all loci except *Est-4, G3pdh-1,* and *Pgm-3* in reproductives. In the latter cases, only one genotype was scored per nest, or individuals were not collected in association with nests and each individual was assumed to originate from a separate nest. Specific numbers of colonies and individuals used to generate genotypic data for each protein locus are presented in table 1.

Eleven of the 13 polymorphic protein loci exhibit codominant patterns of inheritance. Ten of these codominant loci are diallelic in *S. invicta* in the United States, whereas the eleventh, *Est-6,* possesses four alleles, the two rarest of which were pooled for the analyses in this study. The remaining two loci (*Ca-4, Pgm-4*) show banding patterns expected if one or more alleles are interpreted as null alleles (Shoemaker et al. 1993). Identities of allelic electromorphs were in all cases confirmed by running several known standards on each gel. Mendelian inheritance of the products of all 13 loci has been demonstrated by means of family studies (Ross and Fletcher 1985a; Ross 1992; Shoemaker et al. 1992).

Analyses of Electrophoretic Data

Unbiased allele and genotype frequencies were estimated in the study populations by instituting a resampling procedure in which single genotypes (or phenotypes in the case of null loci) were drawn randomly from each nest 200 times (with replacement). This resampling was undertaken to avoid estimating frequencies from sets of nonindependent (nestmate) genotypes, a potential problem in populations with strong family structure (e.g., Crozier et al. 1987). Population allele and genotype frequencies for the codominant markers were estimated as the arithmetic mean frequencies in the 200 resampled genotype distributions. For the null loci, the frequencies of the null alleles were estimated as the square root of the mean frequencies of the null phenotypes. This procedure assumes both that the null alleles are recessive with respect to the banding phenotypes and that the genotypes are in Hardy-Weinberg equilibrium, assumptions that were confirmed in the monogyne population by reestimating allele frequencies from family phenotype distributions using the maximum-likelihood procedure of Halliday (1979) (data from $N = 32$ and $N = 40$ families for *Ca-4* and *Pgm-4,* respectively). The 95% confidence intervals about the allele frequency estimates were constructed for all loci by eliminating the 10 extreme high and low values derived from the 200 resampled distributions. Only single genotypes per colony

TABLE 1. Frequencies of the most common alleles at thirteen polymorphic protein loci in monogyne and polygyne populations of *Solenopsis invicta* from northern Georgia, U.S. The 95% confidence intervals about the estimates are shown in parentheses below the allele frequencies. *N* is the number of nests and *n* the number of individuals studied; these values are identical when only one genotype was scored per nest or when individuals were not collected in association with nests.

Source material	*Aat-2* (EC 2.6.1.1) Worker larva	*Acoh-1* (EC 4.2.1.3) Worker pupa	*Acoh-5* (EC 4.2.1.3) Worker pupa	*Acyl* (EC 3.5.1.14) Adult winged queen gaster	*Ca-4* (EC 4.2.1.1) Worker pupa	*Ddh-1* (EC 1.8.1.4) Worker larva	*Est-4* (EC 3.1.1.–) Adult winged queen mesasoma
Monogyne population	0.902 (0.870–0.935)	0.859 (0.813–0.906)	0.840 (0.783–0.900)	0.873 (0.838–0.919)	0.668 (0.612–0.729)	0.726 (0.671–0.780)	0.550 (0.417–0.667)
N	46	32	30	37	32	41	30
n	1048	900	825	706	917	1169	30
Polygyne population	0.924 (0.862–0.966)	0.826 (0.741–0.897)	0.732 (0.655–0.828)	0.849 (0.776–0.914)	0.780 (0.669–0.871)	0.850 (0.776–0.931)	0.635 (0.517–0.741)
N	29	29	29	29	29	29	29
n	945	951	948	915	944	960	914

TABLE 1. Extended.

Source material	*Est-6** EC 3.1.1.–) Worker pupa	*G3pdh-1* (EC 1.1.1.8) Adult winged queen mesasoma	*Pgm-1* (EC 5.4.2.2) Adult worker mesasoma	*Pgm-3*† (EC 5.4.2.2) Worker pupa	*Pgm-3*† (EC 5.4.2.2) Reproductive queen mesasoma	*Pgm-3*† (EC 5.4.2.2) Adult male mesasoma	*Pgm-4* (EC 5.4.2.2) Worker larva	*Pro-5* (EC –.–.–.–) Adult winged queen mesasoma
Monogyne population	0.357 (0.300–0.417) 0.444 (0.383–0.500)	0.650 (0.517–0.767)	0.878 (0.848–0.902)	0.759 (0.721–0.798)	0.748 (0.721–0.776)	0.815 (0.741–0.888)	0.700 (0.632–0.758)	0.745 (0.700–0.783)
N	30	30	46	52	330	108	40	30
n	886	30	1350	1316	330	108	1224	630
Polygyne population	0.223 (0.138–0.328) 0.602 (0.483–0.707)	0.678 (0.586–0.776)	0.851 (0.759–0.931)	0.556 (0.466–0.672)	0.376 (0.331–0.413)	0.251 (0.182–0.318)	0.466 (0.282–0.634)	0.790 (0.707–0.879)
N	29	29	29	29	80	22	26	29
n	944	919	955	955	1340	112	825	824

* This locus has four alleles; frequencies of the two most common are presented here. The top entry for each population is for the *a* allele and the bottom entry is for the *b* allele.
† Frequencies are for the *a* allele.

were scored for *Est-4, G3pdh-1,* and *Pgm-3* in reproductives from the monogyne population. Thus, in these cases, 95% confidence intervals about the allele frequency estimates were constructed by eliminating the 10 extreme high and low values derived from 200 bootstrap samples drawn from the original data sets (Weir 1990).

The fixation index F_{ST} was estimated for the 11 most common alleles at all the codominant markers except *Pgm-3* (which cannot be assumed to be neutral, see below) as a measure of genetic differentiation between the two study populations. The method of Weir and Cockerham (1984) was used to obtain a single unbiased estimate of F_{ST} and its variance by jackknifing over the 10 loci used.

Genotypes scored for each codominant marker were tested for conformity to Hardy-Weinberg equilibrium (HWE) for each population and for the pooled data from both populations by comparing the genotype proportions in each resampled distribution to proportions expected under HWE (χ^2 tests with Yates's continuity correction, $\alpha = 0.05$; Weir 1990). The probabilities of significant genotypic disequilibrium for each marker were taken as the proportions of the 200 tests in which there were significant departures of the observed genotype ratios from those expected under HWE. Only single tests for conformity of the observed genotypes to HWE were conducted for *Est-4* and *G3pdh-1,* and for *Pgm-3* in queens, in the monogyne population.

Data from Pgm-3

Data for the marker *Pgm-3* from the different life stages, castes, and sexes of both social forms originated from the same colonies used for the other markers, as well as from the following supplemental material (Ross 1992): (1) 52 additional polygyne colonies with their reproductive queens and adult males, (2) 59 additional monogyne colonies with their mother queens, (3) 271 newly mated monogyne queens not associated with colonies, and (4) 108 adult monogyne males collected from as many colonies. The locus *Pgm-3* is unique among the 13 protein loci studied in that it has been shown to be under strong directional selection in the polygyne form but not the monogyne form of *S. invicta* (Ross 1992). The resulting strong allele frequency differences between the two study populations make this an exceptionally useful marker for detecting and measuring interform gene flow, as detailed below.

In addition to obtaining population data for *Pgm-3* from field-collected samples, laboratory progeny studies were conducted for this marker using queens of the polygyne form. These studies were undertaken to reconstruct *Pgm-3* mating types in the polygyne population, with the goal of clarifying the nature of interform gene flow (see also Ross 1992). Sixty-nine naturally mated, wingless queens collected from 25 polygyne nests were established individually in small rearing units with workers and brood in the laboratory. Ten to 13 worker pupae were collected from each unit 6 wk after they were set up, at which time these pupae were known to be the offspring of the isolated queens (e.g., Ross and Fletcher 1985a). The *Pgm-3* genotypes of the brood and their mothers were then determined, and by comparing them, it was possible to infer the *Pgm-3* genotypes of the father of each family [queens from this population typically mate with only a single male (Ross and Fletcher 1985a)]. Thus, the *Pgm-3* mating types giving rise to the 69 families derived from polygyne queens could be reconstructed.

Results

The monogyne and polygyne study populations share identical alleles at each of the 13 polymorphic protein loci. The frequencies of the most common allele at each locus (the two most common at *Est-6*), as well as the 95% confidence intervals for these values, are presented for both populations in table 1. The confidence limits overlap between the polygyne and monogyne forms for all 12 presumably neutral markers (all markers except *Pgm-3*), although the overlap is small for *Ddh-1* and *Pgm-4.* The absence of meaningful genetic differentiation between the forms at these neutral loci is further evident from the estimated value of F_{ST}, which is indistinguishable from zero (0.007 ± 0.009; $F_{ST} \pm$ SE). Allele frequencies differ significantly between the social forms at the locus under strong differential selection, *Pgm-3,* with the difference especially pronounced in reproductive queens, the class of individuals on which selection associated with this locus acts (Ross 1992).

If the two forms are united by gene flow, then the pooled genotypes from the two study populations should conform to Hardy-Weinberg equilibrium (HWE) at those protein loci for which each of the social forms exhibits genotypic equilibrium. Probabilities of significant deviation from HWE at each protein locus are presented

TABLE 2. Probabilities of significant deviation from Hardy-Weinberg genotype proportions at 11 codominant polymorphic protein loci in monogyne and polygyne populations of *Solenopsis invicta* from northern Georgia, United States, and in the pooled data from both populations. Values represent the proportion of 200 resampled genotype distributions (one genotype sampled per nest) that deviated significantly from distributions expected under Hardy-Weinberg equilibrium (see text). For *Est-4, G3pdh-1,* and *Pgm-3* in reproductive queens, only one genotype per nest was scored in the monogyne population; thus, only one test for Hardy-Weinberg equilibrium was conducted. Sample sizes are listed in table 1.

	Aat-2	*Acoh-1*	*Acoh-5*	*Acy1*	*Ddh-1*	*Est-4*	*Est-6*	*G3pdh-1*	*Pgm-1*	*Pgm-3*		*Pro-5*
										Worker pupae	Reproductive queens	
Monogyne population	0	0.005	0	0	0.060	NS* ($P > 0.50$)	0.050	NS* ($P > 0.25$)	0	0.005	NS* ($P > 0.10$)	0.015
Polygyne population	0.010	0	0.005	0.005	0.025	0.020	0.010	0.015	0	0.230	1.0	0
Pooled data	0	0.005	0.015	0	0.140	0	0.020	0.015	0.005	0.025	0.555	0.035

* NS, nonsignificant deviation from Hardy-Weinberg equilibrium for the single test conducted.

for each of the populations, as well as for the pooled data, in table 2. For the monogyne population, all of the markers show a close match between observed and expected genotype proportions, with the highest probability of significant deviation (0.06) found for *Ddh-1* (attributable to heterozygote deficiencies). For the polygyne population, all of the markers except *Pgm-3* also show close concordance between the observed genotype proportions and those expected under HWE. Finally, the pooled population genotype ratios also match Hardy-Weinberg ratios closely, with the exception of *Ddh-1* and *Pgm-3* in reproductive queens. The deviation from HWE for *Ddh-1* in the pooled data stems from a deficiency of heterozygotes (attributable to the combined effects of heterozygote deficiencies in the monogyne form and a small Wahlund effect), whereas the much larger deviations for *Pgm-3* in the polygyne and pooled data are caused by excess heterozygosity. The results of these tests for HWE in the pooled population samples accord well with the data on allelic differentiation between the social forms: the protein markers that are assumed to be neutral typically exhibit allele and genotype frequencies consistent with substantial interform gene flow.

Estimates of the proportions of matched matings by queens (Θ) in the monogyne and polygyne study populations, and of the effective numbers of sex alleles (K) that would give rise to such proportions, are shown in table 3. Estimates of the numbers of alleles are statistically indistinguishable between the social forms, regardless of whether one or two major loci are assumed to determine sex. The similarities in these estimates are best appreciated by reference to a native population of *Solenopsis invicta* in northeastern Argentina, in which values of K are estimated to be 86 (single locus) or 14 (two loci) alleles (Ross et al. 1993). The study populations in Georgia are expected to share similar numbers of sex alleles if they are linked by gene flow.

The marked interform differentiation that exists at the selected protein locus, *Pgm-3,* can be useful for confirming the occurrence of gene flow between the two study populations as well as for elucidating the magnitude and routes of such gene flow. Ross (1992) and Keller and Ross (1993b) demonstrated that negative selection acts on the allele *Pgm-3*^{a} in the polygyne form because queens homozygous for this allele are systematically destroyed by workers as the queens initiate reproduction. The result of this genotype-

TABLE 3. Estimates of the proportions of matched matings by queens and the effective numbers of sex alleles in monogyne and polygyne populations of *Solenopsis invicta* from northern Georgia, United States. The 95% confidence intervals about the estimates are shown in parentheses. The proportion of matched matings is equal to the proportion of diploid-male-producing queens in each population because queens of both social forms effectively mate only once (e.g., Ross and Fletcher 1985a).

	Proportion of matched matings (Θ)	Number of sex alleles (K, single locus)	Number of sex alleles (K, two loci)
Monogyne population	0.164 (0.136–0.193)	12.2 (10.4–14.7)	5.35 (4.99–5.87)
Polygyne population	0.198 (0.134–0.267)	10.0 (7.49–14.9)	4.95 (4.30–5.91)

specific mortality is a far lower frequency of *Pgm-3*^{a} in reproductive queens of the polygyne form than in such queens of the monogyne form. Frequencies of *Pgm-3*^{a} in males of the two forms reflect the strong allele frequency differences found for reproductive queens (table 1), as is expected because haploid males are derived from the unfertilized eggs of their mothers.

High levels of heterozygosity are expected at *Pgm-3* in the female offspring of queens that mate with males of the alternate social form. This is because levels of heterozygosity above those predicted from the Hardy-Weinberg Law and average allele frequencies are generated when allele frequencies differ between the sexes (Hedrick 1985), as they do at *Pgm-3* for males and queens of the different social forms. Furthermore, because *Pgm-3* allele frequencies are known for reproductives of both sexes in both forms, the magnitude of excess heterozygosity at this locus can be used to estimate the extent of interform mating.

Excess heterozygosity is not found at *Pgm-3* among the female offspring of monogyne queens (table 2, monogyne worker pupae). Thus, there is no indication that substantial interform mating occurs between monogyne queens and polygyne males. In contrast, the large excess of *Pgm-3* heterozygotes seen among worker pupae in the polygyne population (table 2) suggests that some fraction of polygyne queens mates with immigrant monogyne males. Assuming that sexuals mate randomly with respect to *Pgm-3* genotype (that is, there is no assortative mating), the extent of this interform mating can be estimated from the formula

$$H_f = q_f + (1 - 2q_f)\cdot(y_M \cdot q_{m(M)} + y_P \cdot q_{m(P)}),$$

where H_f is the heterozygosity of female offspring, q_f is the frequency of allele *Pgm-3*^{a} in reproductive polygyne queens, $q_{m(M)}$ is the frequency of *Pgm-3*^{a} in monogyne males, $q_{m(P)}$ is the frequency of *Pgm-3*^{a} in polygyne males, y_M is the proportion of matings of polygyne queens attributed to monogyne males, and y_P is the proportion attributed to polygyne males (e.g., Hedrick 1985, eq. 2.8). The heterozygosity observed among worker offspring in the polygyne study population ($H_f = 0.630$) is best accounted for when $y_M = 1$ (yielding $H_{f(exp)} = 0.593$), with any decreased value of y_M leading to greater disparity between the expected heterozygosity at *Pgm-3* and that observed. Thus, this analysis suggests that polygyne queens mate exclusively with monogyne males. [An earlier estimate that 80% of polygyne queens mate with monogyne males (Ross 1992) was based on the use of an incorrect version of the above formula (eq. 2.15 of Hedrick 1985).]

An independent means of quantifying this particular route of interform gene flow comes from reconstruction of *Pgm-3* genotypes of the mating pairs that gave rise to polygyne families studied in the laboratory. By comparing offspring genotypes with those of each mother queen, 53 of the 69 families derived from polygyne queens (77%) were determined to have been sired by males with the *Pgm-3*^{a} genotype. The expected proportion (τ_a) of fathers bearing *Pgm-3*^{a} when both polygyne and monogyne males mate with polygyne queens, assuming random mating with respect to *Pgm-3* genotype, is given by the formula

$$\tau_a = (y_M \cdot q_{m(M)}) + (y_P \cdot q_{m(P)}).$$

Solving for $\tau_a = 0.77$ yields $y_M = 0.91$. Thus, these progeny studies agree with the above heterozygosity analysis in suggesting that all or nearly all polygyne queens mate with monogyne males.

DISCUSSION

This study provides several lines of evidence for extensive, ongoing gene flow between a single pair of monogyne and polygyne populations of *Solenopsis invicta* in its introduced range in the

United States. We have found that allele frequencies at 12 presumably neutral, robustly polymorphic protein markers are statistically indistinguishable between the two social forms, leading to an estimate of F_{ST}, a measure of differentiation between the two types of populations, that is essentially zero. As expected, given the similar allele frequencies, observed genotype ratios at codominant protein markers generally match the ratios expected under Hardy-Weinberg equilibrium when data from the two forms are pooled, suggesting that interform gene flow is of sufficient magnitude that this population pair resembles a single panmictic unit genetically. Furthermore, the two study populations share similar numbers of alleles at the sex-determining locus (loci), as would be expected if there is ongoing gene flow between them. The results presented here are compatible with the conclusions of an earlier study of five sets of geographically paired monogyne and polygyne populations that were located throughout the introduced range of *S. invicta* (Ross et al. 1987). That study showed that genetic distances between geographically paired populations of the alternate social forms, as estimated from 26 protein loci, were significantly lower than genetic distances between populations of a single social form paired without regard to location. Together these studies suggest that geographic proximity is the most important determinant of genetic similarity between fire ant populations in the United States, the pattern expected if gene flow occurs between close populations regardless of whether they exhibit identical social organizations.

Although data from the neutral protein markers are consistent with ongoing interform gene flow, they do not demonstrate it. This is because current gene flow cannot be distinguished from retention of ancestral polymorphisms at similar frequencies in descendant populations that have become reproductively isolated only recently. Polygyne populations of *S. invicta* may have arisen repeatedly from neighboring monogyne populations during this ant's invasion of the southeastern United States over the past 60 yr (e.g., Fletcher 1983; Ross et al. 1987). Thus, even if reproductive isolation were conferred instantly with a shift in social organization, there may not have been sufficient time for allele frequencies at the neutral protein markers to diverge significantly through drift if effective population sizes are large (Golding 1992), as they seem to be in introduced *S. invicta* (e.g., Vinson and Greenberg 1986; Porter et al. 1988). Likewise, similar numbers of sex alleles in the two forms might be expected even in the absence of interform gene flow, as long as neither form experienced population bottlenecks during the period since gene flow was abolished.

In contrast to this inherent ambiguity in data from most of the markers, data from the selected locus *Pgm-3* provide compelling evidence that the genetic similarity of the monogyne and polygyne study populations is caused at least in some measure by ongoing gene flow. Maintenance of the allele $Pgm\text{-}3^a$ in the polygyne population despite strong directional selection against it, as well as the consistent excess heterozygosity found at this locus among the offspring of polygyne queens, are best explained by a high influx of $Pgm\text{-}3^a$ via males from the surrounding monogyne population, where this allele is very common. Independent evidence from reconstruction of the *Pgm-3* mating types that gave rise to polygyne families strongly supports the conclusion from the analysis of excess heterozygosity that polygyne queens mate predominantly with males bearing allele $Pgm\text{-}3^a$, that is, predominantly with monogyne males.

The suggestion that gene flow between the social forms is effected by immigration of monogyne males into the polygyne population, followed by their mating with polygyne queens, is reasonable in light of our knowledge of the dispersal and reproductive biology of fire ants and of the operational sex ratios that exist in the different population types. Males of *S. invicta* are capable of long-distance dispersal (Markin et al. 1971); because the polygyne study population is modest in size and is surrounded by the monogyne form (Fletcher 1983), the potential thus exists for many monogyne males to reach polygyne queens. Gene flow occurs only if these dispersing males mate with resident queens, however, raising the question of how immigrant monogyne males outcompete resident polygyne males for such matings. The answer may lie in the fact that the great majority of males produced in polygyne populations is diploid and infertile (see Hung et al. 1974; Ross and Fletcher 1985b; Ross et al. 1993). [Diploid males are absent from the monogyne form because only nests with multiple queens can bear the cost of their production (Ross and Fletcher 1986).] The presence of numerous sterile diploid males creates a strong female bias in the operational sex ratio of the polygyne form, which is estimated to be 1:6 (male : female) in our study population (Vargo and Fletcher 1987; E. L. Vargo, unpubl. data). In contrast, the nu-

merical sex ratio in the adjacent monogyne population is slightly male biased at 1:0.72 (Vargo and Fletcher 1987). The highly female-biased sex ratio in the polygyne form suggests that there are insufficient numbers of fertile haploid males available to compete effectively with immigrant monogyne males for matings with resident queens.

Several lines of evidence indicate that dispersal of monogyne males into polygyne populations, followed by their successful mating, may be the most important or even sole route of interform gene flow in *S. invicta*. Polygyne males are unlikely to effect substantial gene flow by dispersing into monogyne populations and mating with monogyne queens because relatively few fertile males are produced in polygyne populations [many fewer males are produced in polygyne than in monogyne nests and most of these are diploid (Vargo and Fletcher 1987; Ross and Fletcher 1985b; Ross et al. 1993)]. This conclusion is supported by the absence of detectable excess heterozygosity at *Pgm-3* in monogyne offspring females (see table 2 and Ross 1992), such excess heterozygosity being expected if monogyne queens (in which $Pgm\text{-}3^a$ is common) mate with polygyne males (in which $Pgm\text{-}3^a$ is uncommon) to any great extent.

Queens of either form are unlikely to mediate interform gene flow because, though capable of dispersing considerable distances, they are probably unable to establish themselves as reproductives in populations of the alternate social form. Monogyne queens attempting to enter polygyne nests or to establish nests independently in polygyne populations invariably are executed by polygyne workers. This conclusion follows from the observation that reproductive queens homozygous for $Pgm\text{-}3^a$ are completely absent from the polygyne population, even though this is the most common genotype among winged (dispersing) monogyne queens (frequency = 0.476; Ross 1992). Mature winged monogyne queens exhibit far greater fat reserves and a faster pace of reproductive development than mature polygyne queens (Porter et al. 1988; Keller and Ross 1993a), differences that likely parallel differences in the quantities of queen pheromone produced by the two types of queens as they initiate reproduction (Fletcher and Blum 1983; Willer and Fletcher 1986). Polygyne workers, which are intolerant of any queens exhibiting precocious reproductive development (Keller and Ross 1993b), probably use the elevated individual pheromone production of immigrant monogyne queens as a proximate cue to recognize and systematically execute all such queens (e.g., Fletcher and Blum 1983).

Newly mated polygyne queens that disperse into the monogyne population probably also fail in most instances to become established reproductives. Extensive fat reserves are essential for successful independent founding of colonies in ants (Keller and Passera 1989). Thus, the relatively small amount of such reserves in winged polygyne queens implies that they cannot successfully found colonies independently (Porter et al. 1988) and that they must instead enter established nests to survive and reproduce. Evidence suggests that newly mated polygyne queens are indeed adopted into polygyne nests (Glancey and Lofgren 1988; Porter 1991), whereas they are not normally accepted into queenright monogyne nests (e.g., Fletcher and Blum 1983). If nests in the monogyne population were commonly derived from polygyne foundresses, then excess heterozygosity would be generated at *Pgm-3* among progeny females in this population (given the strong allele frequency differences between polygyne queens and monogyne males, assuming that dispersal of polygyne queens is random with respect to *Pgm-3* genotype, and assuming that most of these dispersalists mate with monogyne males). Again, no excess heterozygosity has been detected at *Pgm-3* in the monogyne population.

A recent report by Dunton et al. (1991) describes two enzyme electromorphs present in queens of the polygyne form but not the monogyne form of *S. invicta* in Texas, United States. The electromorphs unique to the polygyne form have not been demonstrated to be allelic to electromorphs present in the monogyne form; thus, insofar as their genetic bases are not understood, their distributions cannot address the question of interform gene flow. A host of biological differences distinguish the two forms—morphological, behavioral, and physiological (Fletcher et al. 1980; Greenberg et al. 1985; Vargo and Fletcher 1987, 1989; Vargo and Ross 1989; Keller and Ross 1993a)—most of which appear to be secondary consequences of the most fundamental difference, colony queen number. No evidence exists to suggest that any of these trait differences have a genetic basis; rather, they seem to be determined by differences in the social and pheromonal environments in nests of the two forms.

Our study indicates that, contrary to previous hypotheses (e.g., Ross and Fletcher 1985a), the two social forms of *S. invicta* in the United States

are linked by extensive gene flow. Thus, the social forms do not represent an intermediate stage of divergence in the sense of having well-developed reproductive isolation, and their relevance as a model for speciation driven by social evolution might therefore be questioned. On the other hand, the shift to an alternative social organization clearly has had an effect on the mode of gene flow, constraining it to be unidirectional and mediated through one sex only. These constraints arise as a direct consequence of the differing social environments that characterize the two forms. The presence of multiple reproductive queens in polygyne nests leads to the production both of new queens that are physiologically ill-equipped to survive in monogyne populations and of workers that are intolerant of queens with the monogyne physiological phenotype. The apparent result is an inability of females of either form to mediate interform gene flow. The presence of multiple queens in polygyne nests dictates the pattern of male-mediated gene flow as well. The production of many infertile (diploid) males in the polygyne but not the monogyne form skews the polygyne sex ratio to an extent that many receptive polygyne queens are available for mating with immigrant monogyne males, whereas few fertile (haploid) polygyne males are available to emigrate. Thus, gene flow via males occurs only from the monogyne to the polygyne form in the case of our study populations. Future work will focus on native population pairs in South America, where male diploidy is relatively rare (Ross et al. 1993). A more balanced operational sex ratio in native polygyne fire ants may mean that this form is less "dependent" on the monogyne form to provide fertile males, perhaps restricting the magnitude of interform gene flow occurring by this route.

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